

BEHAVIORAL RESPONSES OF CATNIP (*NEPETA CATARIA*) BY TWO SPECIES OF MOSQUITOES, *Aedes aegypti* AND *ANOPHELES HARRISONI*, IN THAILAND

SUPPALUCK POLSOMBOON,¹ JOHN P. GRIECO,² NICOLE L. ACHEE,²
KAMLESH R. CHAUHAN,³ SOMCHAI TANASINCHAYAKUL,⁴ JINRAPA POTHIKASIKORN⁵
AND THEERAPHAP CHAREONVIRIYAPHAP^{1,6}

ABSTRACT. An investigation of the biological effect of catnip oil (*Nepeta cataria* L.) on the behavioral response of field collected *Aedes aegypti* and *Anopheles harrisoni* was conducted using an automated excito-repellency test system. *Aedes aegypti* showed significantly higher escape rates from the contact chamber at 5% catnip oil compared to other concentrations ($P < 0.05$). With *Anopheles harrisoni*, a high escape response was seen at 2.5% catnip oil from the contact chamber, while in the noncontact chamber a higher escape response was observed at a concentration of 5%. Results showed that this compound exhibits both irritant and repellent actions.

KEY WORDS Behavioral responses, irritancy, repellency, *Aedes aegypti*, *Anopheles harrisoni*, catnip

INTRODUCTION

Many areas of the world are at risk for a wide variety of arthropod-borne diseases with millions cases occurring each year (WHO 2007). A significant growth in human population, demographic movement from rural to more crowded urban areas, and an increase in tourism-based facilities have contributed to an increasing trend in disease transmission. Prevention of these diseases remains almost entirely dependent on various methods of vector control. Control of vectors by insecticides remains the most important means of reducing disease transmission and protection from mosquito bites (Roberts et al. 1997).

Chemicals protect humans from the bite of mosquitoes through 3 different actions: irritation after making contact, repelling prior to contact, or by killing the insects (Grieco et al. 2007). Most research has focused on the toxic function of chemicals, whereas comparatively few studies have concentrated on nontoxic chemical actions. Nontoxic action can be categorized into 2 distinct mechanisms, contact irritancy and noncontact repellency. Irritant responses result from physical

contact with chemical-treated surfaces, whereas repellency is an avoidance response devoid of making actual contact with the chemical (Char-eonviriyaphap et al. 1997, Roberts et al. 1997). Much of the early research on behavioral responses was concentrated on the synthetic chemicals (Pothikasikorn et al. 2007). In Thailand synthetic compounds, including organophosphates, carbamates, and pyrethroids, have been used with varying degrees of success in national public health vector control programs (Reiter and Gubler 1997). Since 1994 the Ministry of Public Health (MOPH) in Thailand has recommended the use of deltamethrin in public health to control malaria and dengue haemorrhagic fever. Recent studies have reported the spread of deltamethrin resistance in several field *Culex quinquefasciatus* Say and *Aedes aegypti* L. populations from Thailand (Somboon et al. 2003). Alternative compounds or new methods of controlling mosquito vectors are needed. One source of alternatives lies in botanical compounds that are commonly used as “insect repellents.” These compounds are effective, safe, and increasingly available for domestic use against indoor and outdoor biting mosquitoes and arthropod pests.

One option for preventing the transmission of a vector-borne pathogen to a host is the use of a topical insect repellent. N, N-diethyl-3-methylbenzamide (DEET), that is effective in protecting humans from mosquito bites (Qiu and McCall 1998). Recently several botanical extracts, such as eucalyptus (*Eucalyptus citriodora* Hook), citronella grass (*Cymbopogon nardus* Rendle), thyme (*Thymus vulgaris* L.), clove (*Syzygium aromaticum* L.), and catnip (*Nepeta cataria* L.), were tested as alternative topical mosquito repellents (Barnard 1999, Zhu et al. 2006). Among these the essential oil from catnip proved to be a safe and promising insect repellent. This oil contains 2

¹ Department of Entomology, Faculty of Agriculture, Kasetsart University, Bangkok 10900, Thailand.

² Department of Preventive Medicine and Biometrics, Uniformed Services University of Health Sciences, Bethesda, MD 20814.

³ Invasive Insect Biocontrol and Behavior Laboratory, Plant Science Institute, USDA-ARS, Beltsville, MD 20705.

⁴ Department of Entomology, Faculty of Agriculture, Kasetsart University, Kamphaengsean Campus, Nakhon Pathom 73140, Thailand.

⁵ Department of Microbiology, Faculty of Science, Mahidol University, Bangkok 10400, Thailand.

⁶ To whom correspondence should be addressed.

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stereoisomers of nepetalactone (E,Z and Z,E isomer). The 2 stereoisomers have been reported to function as insect repellents against 13 families of insects (Eisner 1964). The E,Z-nepetalactone form showed to be a stronger repellent against the German cockroach (*Blattella germanica* L.) than the Z,E-nepetalactone one (Peterson et al. 2002). Catnip oil was also reported to be a good repellent for short-term action against house flies and American cockroaches (Schultz et al. 2004). Additionally, catnip oil was found to be a good spatial repellent compound in protecting humans from mosquito bites for at least 6 h posttreatment (Bernier et al. 2005, Zhu et al. 2006). However, no investigation has been performed to identify the 2 distinct categories of behavioral responses, irritancy and repellency, of mosquitoes to catnip oil. We investigated the activity of catnip oil against 2 species of mosquitoes, *Ae. aegypti*, a vector of dengue, and *Anopheles harrisoni* Harbach and Manguin, a vector of malaria in Thailand. Irritant and repellent responses were quantitatively assessed using an automated excito-repellency (ER) test system (Tanasinchayakul et al. 2006).

MATERIALS AND METHODS

Mosquito populations

Aedes aegypti was established from immature stages collected from Pu Teuy Village, Sai Yok District, Kanchanaburi Province (14°17'N, 99°17'E), ~100 km northwest of Bangkok, Thailand, between July and August 2006. Only F-1 generation adults were used in the study. *Anopheles harrisoni* was collected by cow bait from 1800 to 2400 h from the same location between April and September 2006. For cow-baited collections, one cow was placed in a net trap, and mosquitoes were collected from inside the net for 15 min/h. The captured mosquitoes were kept in mosquito cups and provided with 10% sugar solution. *Anopheles harrisoni* was identified the following morning, using the morphological keys of Rattanarithikul et al. (2006).

Mosquito conditioning

Unfed 3- to 5-day-old female *Ae. aegypti* were used in this study. All female mosquitoes were deprived of sucrose solution and water 12 h before testing. With *Anopheles harrisoni*, only field-collected mosquitoes were used for testing, and they were not starved because they were active and host-seeking at time of capture.

Insecticide-impregnated papers

Different concentrations (1%, 2.5%, 5%, and 10%) of essential oil from catnip were impreg-

nated onto test papers, measuring 12 × 15 cm for susceptibility tests and 15 × 17.5 cm for excito-repellency test, following the standard World Health Organization procedure (WHO 1998). Catnip oil was received from the Chemicals Affecting Insect Behavior Lab, United States Department of Agriculture, Beltsville, MD. Nepetalactones (E,Z ~48% and Z,E ~40% isomers) and β -caryophyllene (~9%) are the major constituents in catnip oil. The E,Z and Z,E nepetalactone isomers were 99% chemically pure and 95–98% stereo-chemically pure according to capillary gas-liquid chromatography (Chauhan and Zhang 2004). The structures of nepetalactone isomers were confirmed by gas chromatography (GC) mass spectroscopy and nuclear magnetic resonance spectral analysis (Eisenbraun et al. 1980). Racemic nepetalactone was formulated by mixing 1:1 ratio of E,Z and Z,E nepetalactones, and homogeneity was confirmed by GC.

Dose response assay

The standard WHO tarsal contact test was used in this study. For each test, 5 cylinders (2 for controls and 3 for treatments) were used. Control cylinders contained filter paper impregnated with solvent (acetone), whereas treatments contained filter paper impregnated with the different concentrations of catnip oil in solvent. For each test population, 25 female mosquitoes were exposed for 1 h to catnip oil. Following test and control exposures, knockdown was recorded, and all mosquitoes transferred to separate clean holding containers and provided with 10% sucrose solution. Total knockdown and mortality was recorded after 24 h post-exposure. Each matched test-control series was repeated 4 times per test population.

Excito-repellency tests

In this study we used an automated field excito-repellency test system as described in a recent publication (Tanasinchayakul et al. 2006). Immediately following the 30-min exposure, the number of dead or knockdown specimens remaining inside the chamber, and those that had escaped into the receiving cage, were recorded for each of the 4 chambers. Also, all live specimens that had escaped or remained inside the test chamber were transferred to clean holding cups and provided with a 10% sucrose solution. All test mosquitoes were maintained separately in lots for 24 h postexposure, at which time mortality was recorded.

Data analysis

In contact susceptibility tests, control mortalities exceeding 5% were corrected and adjusted

Table 1. Percentage mortality of *Ae. aegypti* and *An. harrisoni* populations from Kanchanaburi exposed to different concentrations of catnip oil using standard WHO susceptibility test procedures.

Mosquito	Concentration	No. tested	% KD ¹	% mortality \pm SE
<i>Ae. aegypti</i>	1%	100	0	0
	5%	100	4	0
	10%	100	43	3 \pm 0.75
<i>An. harrisoni</i>	1%	100	0	0
	2.5%	100	3	3 \pm 0.48
	5%	100	55	7 \pm 0.63

¹ KD = knock down.

for determining baseline susceptibility in each test population (Abbott 1925). For excito-repellency data, a life table survival analysis approach was used to estimate mosquito escape rates and compared differences in mosquito escape rates between test populations and insecticides (Roberts et al. 1997). Survival analysis provides a robust statistical treatment of sequential excito-repellency data, and relative to other quantitative methods it describes behavioral avoidance. The survival curves minimize loss of valuable information while estimating temporal mosquito escape probability (Roberts et al. 1997). The time in minutes for 25%, 50%, and 75% of the test population to escape was estimated using life table analysis, and these estimates were used as the "escape time" summary statistics (ET₂₅, ET₅₀, and ET₇₅).

A log-rank method was used to compare patterns of escape behavior. This test is designed to detect differences between survival curves that result when the death (or escape) rate in one group is consistently higher than the corresponding rate in the second group, and the ratio is consistent over time. With excito-repellency data, the basic idea underlying the log-rank test involves examining escape observations by 1-min intervals. The log-rank method was proposed by Mantel and Haenzel (1959). The discriminating level for statistical significance was set at 0.05%.

RESULTS

Dose response assay

Bioassays were conducted to obtain the dose response mortality on test populations of 2 mosquito species (*Ae. aegypti* and *An. harrisoni*), collected from Kanchaburi Province, western Thailand, using the WHO susceptible test for adult mosquitoes (WHO 1998). From preliminary screening, 3 concentrations of catnip oil (1%, 5%, and 10% for *Ae. aegypti* and 1%, 2.5%, and 5% for *An. harrisoni*) were selected for the bioassay and behavioral assay. Catnip oil exhibited low toxicity for the 2 test populations (Table 1). Percentage mortality of 2 test populations was comparatively low, regardless of test concentrations. Mortality varied between 0% and 3% for *Ae. aegypti* and 0% and 7% for *An. harrisoni* (Table 1). With *Ae. aegypti*, 94% knockdown at 1 h was observed at 5% catnip oil and a 43% knockdown at 10% catnip oil, whereas a 55% knockdown of *An. harrisoni* was observed at 5% catnip oil.

Excito-repellency test

Data on percentage escape responses of the 2 test populations exposed to different concentrations of catnip oil were recorded in contact and noncontact trials (Tables 2 and 3). With *Ae. aegypti* in contact trials, the greatest escape

Table 2. Escape response and percentage mortality of female *Ae. aegypti* from Kanchanaburi after contact and noncontact with catnip oil in excito-repellency tests.

Conditions	Concentration	Treatment chamber		% KD ¹		Control chamber		% mortality			
		No. tested	% esc. ²	Esc.	Not esc.	No. tested	% esc.	Treatment		Control	
								Esc.	Not esc.	Esc.	Not esc.
Contact	1%	60	35.00	0	0	56	21.43	0	0	0	0
	5%	55	80.00	.81	18.18	58	13.79	2.27	0	0	0
	10%	58	56.90	21.21	40.00	58	18.97	3.03	8.00	0	0
Noncontact	1%	58	31.03	0	0	57	14.04	0	0	0	0
	5%	55	40.00	0	9.09	59	10.17	0	0	0	0
	10%	56	53.57	6.67	34.61	59	11.86	0	0	0	0

¹ KD = knock down.² Esc. = escaped mosquitoes; not esc. = not escaped mosquitoes.

Table 3. Escape response and percentage mortality of female *An. harrisoni* from Kanchanaburi after contact and noncontact with catnip oil in excito-repellency tests.

Condition	Concentration	Treatment chamber		% KD ¹		Control chamber		% mortality			
		No. tested	% esc. ²	Esc.	Not esc.	No. tested	% esc.	Treatment		Control	
								Esc.	Not esc.	Esc.	Not esc.
Contact	1%	59	16.95	0	0	56	1.79	0	2.04	0	0
	2.5%	59	71.19	11.36	18.18	59	8.47	9.52	17.64	0	0
	5%	8	58.62	35.29	62.50	58	8.62	14.70	20.83	0	0
Noncontact	1%	60	15.00	0	0	55	1.82	0	1.96	0	0
	2.5%	57	63.16	0	9.09	58	10.34	2.78	14.04	0	0
	5%	56	67.86	5.26	38.89	54	5.56	10.53	16.67	0	0

¹ KD = knock down.
² Esc. = escaped mosquitoes; not esc. = not escaped mosquitoes.

responses (80%) were observed at 5% catnip oil, whereas the lowest escape responses (35%) were observed from 1% catnip oil. At the highest concentration (10%), a high percentage of knock-down of the test insects was observed from those that had escaped (21.21%) and those that remained in the test chamber (40%). In noncontact trials the highest escape responses were observed at 10% catnip oil (53.57%) and the lowest at 1% catnip oil (31.03%). Percentage knockdown was not as high as that observed from the contact trials. The highest knockdown rate (34.61%) was seen in the nonescaped specimens exposed to 10% catnip oil, whereas a comparatively low knockdown (0–6.67%) was noted in females that had escaped. Within contact trials, a marked escape response of 71.19%, 58.62%, and 16.95% was observed *An. harrisoni* exposed to 2.5%, 5.0%, and 1.0% catnip oil, respectively. In noncontact trials escape responses of this species were comparatively high at 2.5% catnip oil (63.16%) and 5% catnip oil (67.87%) compared to 1% catnip oil (15%). In general, high percentage knockdown was observed at the higher concentrations of catnip oil. Contact trials produced higher numbers of knockdown specimens than those from noncontact trials. The greatest percent of knockdown was observed in

females failing to escape at 5% catnip oil in contact trials (62.50%).

The 24 h mortalities of *Ae. aegypti* and *An. harrisoni* females after exposure to catnip oil in contact and noncontact trials are given in Tables 2 and 3. Lower mortality rates were recorded for *Ae. aegypti* as compared to *An. harrisoni* tested against different concentrations of the catnip oil. With *Ae. aegypti* in contact trials, percentage mortalities of escape and nonescape females varied from 0% to 8%. No mortality was observed from noncontact trials for all test concentrations (Table 2). With *An. harrisoni* in contact trials, the percentage mortality of nonescaping females was high (2.04–20.83%) compared to escaping females (9.52–14.70%). Similarly, high mortality rates were observed from noncontact trials in both escaping and nonescaping females, ranging from 2.78% to 10.53% for escaping and 1.96% to 16.67% for nonescaping females (Table 3).

Escape times (ETs) from chambers treated with different concentrations of catnip oil, measured at 1-min intervals, were designated based on the percentage of the test population escaping, 25% (ET25), 50% (ET50), and 75% (ET75), the treated chamber within 30 min (Table 4). The *Ae. aegypti* test population exposed to the 1%

Table 4. Escape time (ET) in minutes for 25%, 50%, and 75% of 2 species of field mosquito to escape treated chambers with catnip oil (*N. cataria*).

Mosquitoes	Concentration	Contact			Noncontact		
		ET 25	ET 50	ET 75	ET 25	ET 50	ET 75
<i>Ae. aegypti</i>	1%	15	— ¹	—	18	—	—
	5%	1	4	16	8	—	—
	10%	2	16	—	3	20	—
<i>An. harrisoni</i>	1%	—	—	—	—	—	—
	2.5%	4	9	—	3	11	—
	5%	4	14	—	6	12	—

¹ Very few mosquitoes escaped from exposure chambers so that the ET values could not be estimated for a 30-min exposure period.

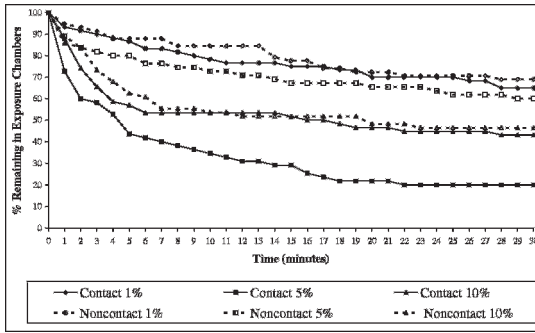


Fig. 1. Comparison of escape pattern of female *Ae. aegypti* from Kanchanaburi in contact and noncontact trials exposed to different concentrations of catnip oil.

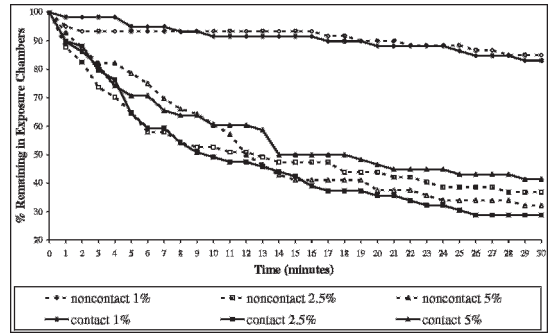


Fig. 2. Comparison of escape pattern of female *An. harrisoni* from Kanchanaburi in contact and noncontact trials exposed to different concentrations of catnip oil.

catnip oil had an ET25 value of 15 min in contact trials and of 18 min in noncontact trials. The ET25 value for *An. harrisoni* in both contact and noncontact trials could not be calculated because of the lack of mosquito movement. At 2.5% catnip oil, the ET25 and ET50 for *An. harrisoni* were 4 and 9 min, in contact trials and 3 and 11 min in noncontact trials, respectively. At 5% catnip oil, the ET25 value was 2 min for *Ae. aegypti* and 4 min for *An. harrisoni* in contact trials, whereas such values in noncontact trials were 8 and 6 min, respectively. The ET50 value was also low (4 min) for *Ae. aegypti*, whereas it was comparatively high for *An. harrisoni* in contact (14 min) and noncontact trials (12 min) (Table 4). At 10% catnip oil, *Ae. aegypti* had a low ET25 values of 2 min in contact trials and 3 min in noncontact trials, but ET 50 values of 16 and 20 min in contact and noncontact trials, respectively. The ET75 values in both contact and noncontact trials at different concentrations of catnip oil could not be estimated because of too few specimens departing the exposure chamber (Table 4).

Contact versus noncontact escape responses of *Ae. aegypti* to 1%, 5%, and 10% catnip oil were compared. Escape probabilities in contact and noncontact trials were significantly higher than in controls for all cases ($P < 0.05$), except for 1% catnip oil when the contact trials were not significantly different from the control. Significant differences in escape responses were observed in 5% catnip oil between contact and noncontact trials ($P < 0.05$). No significant differences in escape response of *An. harrisoni* were observed in all pairs when contact trials was compared to noncontact trial, regardless of the test concentration ($P > 0.05$). Statistically significant differences in escape responses were observed at 2.5% and 5% catnip oil when control was compared to contact and noncontact trials.

Statistical comparisons between concentrations of catnip oil (1%, 5%, and 10% for *Ae. aegypti* and 1%, 2.5%, and 5% for *An. harrisoni*) in

contact and noncontact trials demonstrated that there were significant differences between all pairs ($P < 0.05$), except for catnip oil at 2.5% and 5% against *An. harrisoni* ($P > 0.05$).

The proportions of mosquitoes remaining in the exposure chambers at different test concentrations were used to compare escape probabilities between contact and noncontact trials for *Ae. aegypti* (Fig. 1) and *An. harrisoni* (Fig. 2). A higher escape response of *Ae. aegypti* was observed when exposed to 5% catnip oil in contact trials compared to noncontact trials. Significantly lower escape responses were found at 1% and 10% catnip oil in both contact and noncontact trials when tested against *Ae. aegypti*. The patterns of escaped females of *An. harrisoni* were significantly greater at 2.5% and 5% catnip oil than at 1% catnip oil.

DISCUSSION

Understanding the behavioral responses of mosquito vectors, especially avoidance behavior to test compounds, is of paramount importance to any mosquito control program. There have been numerous attempts to accurately measure the behavioral responses of mosquitoes to insecticides using several types of excito-repency test system (Sungvorunyothin et al. 2001). Because of the inherent complexities of accurately measuring excito-repency in mosquitoes, no test method had been adequate and fully accepted. No test recommended by the WHO will discriminate between the 2 types of behavioral responses, contact irritancy, and noncontact repellency (Roberts et al. 1984). However, an experimental test system described by Roberts et al. (1997) addresses a number of deficiencies attributed to behavioral test systems. This test system was first used to test the avoidance behavior of *An. abimanius* from Belize, Central America (Charonviriyaphap et al. 1997). This prototype test system has been modified further into the collapsible chamber designed for the greater ease

of use and has proved valuable in the evaluation of behavioral responses in several laboratory and field populations of mosquitoes in Thailand and Indonesia (Pothikasikorn et al. 2007). However, this system was still cumbersome and required a minimum of 2 investigators to observe and record data during the 30-min testing period.

Recently an assay for evaluating the 3 types of chemical actions, contact irritancy, spatial repellency, and toxicity, in adult mosquitoes was developed (Grieco et al. 2007), but this system was not designed as a field-adaptable assay. To overcome these technical problems when conducting field studies, a more compatible design was developed and is referred to as an "automated, field-compatible device for testing excito-repellency behavior" (Tanasinchayakul et al. 2006). This system consists of 2 major modifications from the previous model: a substantial reduction in the size of the test box and the use of an electronic sensor for automated counting of mosquitoes as they departed the test chamber through the opened gate into the external holding box. This device has been successfully used to measure the behavioral responses of *Ae. aegypti* from Bangkok, Thailand, to deltamethrin (Tanasinchayakul et al. 2006). Moreover, an automated excito-repellency test system makes it easier for automatically counting escaping mosquitoes from the chamber and recording data by a computer system. This system can eliminate error from confounding factors by human such as human odor, body heat, and carbon dioxide. An additional advantage is the system requires only one investigator to observe and collect escaped mosquitoes from the receiving cage.

In this study we observed the behavioral responses of 2 field-collected mosquito species, *Ae. aegypti* and *An. harrisoni*, collected from Kanchanaburi, western Thailand, to catnip oil, a promising plant derived compound from catnip (Peterson and Coats 2001).

Chemicals protect human from the bite of mosquitoes in 3 different ways: irritate, repel, or kill the mosquitoes (Grieco et al. 2007). In this study *Ae. aegypti* demonstrated clear behavioral escape responses to catnip oil in both contact and noncontact trials compared to the control trials. Greater contact irritancy escape responses from 5% catnip oil were documented in *Ae. aegypti*, compared with 1% and 10% catnip oil. All tests showed mosquitoes successfully departed treated surfaces and chambers before receiving a lethal dose of test compound. Higher knockdown rates were observed at the higher doses, regardless of test condition, indicating a strong vapor from the test chemical affected the test specimens. However, a high percentage of recovery (>92%) was observed, indicating no toxic action of catnip oil. Recently several studies examined the repellency effect of catnip oil in mosquito species and other

insects (Peterson and Coats 2001; Schultz et al. 2004; Bernier et al. 2005; Chauhan et al. 2005; Webb and Russell 2007; Zhu et al. 2006). With *An. harrisoni*, contact irritancy and noncontact repellency were quite high, especially at 2.5% catnip. Knockdown rates were somewhat greater at the higher concentrations with greater percentage mortality of both contact and noncontact mosquitoes, suggesting *An. harrisoni* were more sensitive to the toxic action of catnip oil.

The protection time of catnip oil has been reported elsewhere. Catnip oil was shown to be an effective repellent up to 6 h against *Ae. albopictus* (Zhu et al. 2006). In Australia catnip oil demonstrated mean protection times, ranging from 0 min for *Ae. aegypti* up to 240 ± 60 min for *Cx. quinquefasciatus* (Webb and Russell 2007). In contrast, catnip oil showed a long protection time to *Ae. vigilax*, *Cx. annulirostris*, and *Cx. quinquefasciatus* compared to other potential natural plant extracts (Webb and Russell 2007). In our study the protection time of catnip oil on mosquito populations was not evaluated. However, we found that catnip oil has strong irritant and repellent actions on mosquito test populations as indicated by the comparatively low escape time.

In summary, several studies have investigated mosquitoes repellents derived from plant extracts (Suwonkerd and Tantrarongroj 1994), but none have described contact irritant and noncontact repellent actions. With the existence of a field-automated excito-repellency test system, the 2 behavioral actions of catnip oil on 2 field-collected mosquito species were quantified. The resulting data will help in better understanding how catnip oils act against mosquitoes and how they might be used in the future.

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